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THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

JAN 23 1974

Application for Research Grant (Revised) Date: 1/17/74

(Use extra pages as needed)

1. Principal Investigator (give title and degrees):

Joseph C. Arcos and Mary F. Argus
Professor of Medicine Professor of Medicine
L. Chem., Ch. E., D. Sc. B. S., M. S., Ph. D.

Mailing address:

Seamen's Memorial
Research Laboratory
USPHS Hospital
210 State Street
New Orleans, La. 70118

2. Institution & address:

Tulane University School of Medicine
1430 Tulane Ave.
New Orleans, La. 70112

3. Department(s) where research will be done or collaboration provided:

Department of Medicine

4. Short title of study: Synergistic effects of polycyclic hydrocarbons and nitrosamines in pulmonary carcinogenesis. Potential repressors of metabolic activation of nitrosamines.

5. Proposed starting date: 3/1/74

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

First, to explore experimentally the very definite possibility (see 8. Background and brief statement of working hypothesis) that a significant proportion of the lung tumor incidence of cigarette smokers is due to synergism between hydrocarbons and nitrosamines present in the smoke, and to attempt to elucidate the mechanism of this synergism by determining how the enzyme systems metabolizing these two types of compounds are related to each other. A further ramification of such studies is that the oral nitrosamine intake of smokers (as dietary nitrosamine contaminants, or pro-nitrosamine additives, such as nitrite together with ubiquitously present secondary amines in meat products) could play an important role in their high lung cancer incidence.

Second, to investigate whether certain nontoxic inhibitors of nitrosamine metabolism — a process known to be required for carcinogenesis by these agents — also inhibit nitrosamine carcinogenesis and decrease lung tumor incidence by disrupting the hydrocarbon-nitrosamine synergism.

In the present study we propose to investigate the synergism toward lung tissue of the highly carcinogenic hydrocarbon, 3-methylcholanthrene (MC), and the carcinogenic nitrosamine, dimethylnitrosamine (DMN). We will determine the combined carcinogenic effect of the two compounds in an inbred strain of mice in which aryl hydrocarbon hydroxylase (AHH), the enzyme system which both activates and detoxifies MC, is present or inducible, and in a

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strain of mice in which this enzyme system is noninducible. Throughout the time course of this study, the effect of administration of the compounds on the tissue level of AHH and the level of DMN-demethylase, a mixed function oxidase system capable of converting DMN to a proximate carcinogen, will be determined. If the synergism is established to be strain dependent, the inheritability of the susceptibility will be determined in first generation hybrid crosses of the two strains. Also within the framework of the present study we intend to determine the inhibitory effect of β-naphthoflavone and pregnenolone-16α-carbonitrile on the carcinogenic activity of DMN; these two agents are powerful inhibitors of DMN metabolism, and are virtually nontoxic:

8. Background and brief statement of working hypothesis:

A.) Hydrocarbon-nitrosamine synergism. — A previous study (1) in our laboratory has shown that administration of 3-methylcholanthrene (MC) inhibits liver tumor induction in rats by simultaneously administered dimethylnitrosamine (DMN). In a series of investigations (2-6) we have established that this is due to inhibition in the liver of biosynthesis of the enzyme, DMN--demethylase, the key enzyme transforming this nitrosamine to its actual carcinogenic metabolite. In fact DMN-demethylase has proved to be one of the rare mixed function oxidases, the de novo synthesis of which is repressed by MC, phenobarbital and numerous other so-called "enzyme inducers". By studies with 58 polynuclear hydrocarbons we have established that the repression of DMN-demethylase synthesis, just as the previously-studied induction of other microsomal enzyme systems (7), is a function of molecular size and planarity; and what is most striking, these two effects show a mirror image relationship (5, 6). This finding, together with the fact that a much shorter latency is required for enzyme induction than for enzyme repression (e.g., 5-6 hours vs. 12-13 hours with MC), provide support for our previously suggested theoretical model that inducible and repressible microsomal oxidase levels are regulated by cascade-coupled operons (8).

An unexpected and important finding of the carcinogenesis study (1) was that while the hepatocarcinogenicity of DMN is inhibited by the simultaneous oral administration of MC, the two agents have a powerful synergistic effect in lung tumor induction. Indeed, whereas at the levels used in the study neither the nitrosamine nor the hydrocarbon alone was carcinogenic in the lung, combined administration of the two agents brought about a 15% pulmonary tumor incidence. More recently Cardesa et al. (9) have demonstrated a syncarcinogenic effect with MC and DMN in the lungs of Swiss mice. The practical significance of these results lies in the facts (a) that MC is known to be very similar or identical in action to the two potent hydrocarbon carcinogens, 3,4-benzopyrene and 3,4-benzofluoranthene, present in cigarette smoke (10), and (b) that DMN, methylethylnitrosamine, and N-nitrosopiperidine are also present in the smoke (11) at a total level of similar order of magnitude as the hydrocarbons (12).

The above considerations point to the high probability that a significant proportion of the lung tumor incidence of smokers is due to synergism between carcinogenic hydrocarbons and nitrosamines present in the smoke, rather than to hydrocarbons alone. When DMN is administered to rats at a higher total dose than in the above-cited study (1), lung tumors are produced

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by DMN alone (13). Thus since the lung is a tissue target of DMN, it is not unreasonable to suppose that administration of DMN brings about deletion of various microsomal mixed function oxidases in that tissue just as is known to happen in the liver (14). This would result in a decrease of the rate of MC hydroxylation via AHH in the lung, whereby higher topical level of hydrocarbon would become available for tumorigenesis. Another possible explanation is that DMN induces AHH synthesis and thus the rate of MC activation is accelerated. These possibilities will be explored by measuring the rate of in vitro MC metabolism in lung tissue during the time-course of prolonged DMN and MC administration.

On the other hand the possibility can not be ignored that repressibility of DMN-demethylase in the lung may be greatly decreased in the adult animals in which the carcinogenicity studies have been carried out, as opposed to the response in weanling animals which have been used in the enzyme studies. It is well known that the inducibility of the synthesis of various hepatic mixed function oxidases by polycyclic hydrocarbons gradually decreases as the animals progress from the weanling to the adult stage. The requirement regarding age for inducibility by hydrocarbons in lung tissue does not appear to have been studied. In the absence of proof to the contrary it cannot be entirely excluded at present that in lung tissue the repressibility of DMN-demethylase may entirely disappear with animal age. The possibility exists that DMN-demethylase synthesis in the lung may even be induced by prolonged ingestion of MC in adult animals. Thus the level of DMN-demethylase activity will also be determined in lung tissue during the time course of prolonged DMN and MC administration.

The lung tissue for these enzyme (AHH and DMN-demethylase) activity determinations will be provided by animals treated with MC and DMN in groups paralleling a study of the syncarcinogenic effects of these two compounds toward the lung. Animal test systems having very special characteristics to aid in elucidating relationships between susceptibility to chemical carcinogenesis and ability to metabolize enzymatically chemical carcinogens have recently been defined. It has been established that induction or increase in AHH activity occurs only in certain inbred strains of mice (15, 16) and these strains are more susceptible to MC-induced subcutaneous tumor than are strains in which AHH is noninducible (17, 18). For example, the C57BL/6 (B6) mouse strain in which the hepatic AHH is inducible in all mice gave a 83% tumor incidence for MC-induced subcutaneous tumors, while the DBA/2 (D2) strain in which hepatic AHH is noninducible in all mice gave only a 7% tumor incidence (18). It was further shown that the enzyme induction segregated as a single autosomal dominant gene in crosses of strains C57BL/6 and DBA/2 (15, 16), and that the genetically controlled presence of the inducibility of AHH activity in backcrosses between C57BL/6 and DBA/2 strains correlated with susceptibility to MC-induced subcutaneous tumors.

Because of these unique characteristics of these two strains of mice (C57BL/6 and DBA/2) we plan to employ them in the presently proposed studies on the possible synergism between hydrocarbon and nitrosamine carcinogens (specifically MC and DMN) on lung tumorigenesis. These strains will

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especially lend themselves to an attempt to elucidate (a) the mechanism of such a synergism, (b) how it is related to induction (or repression) of the enzyme systems involved with metabolism of the carcinogenic agents, (c) how these enzyme systems themselves are interrelated, (d) to provide evidence for or against our hypothesis that inducible and repressible microsomal oxidase levels are gene regulated by cascade-coupled operons, and (e) to indicate inheritability of susceptibility to the action of certain agents and combinations of agents.

B.) Anti-carcinogenic effects of β-naphthoflavone and pregnenolone-16α-carbonitrile. — We have demonstrated that not only polycyclic hydrocarbons but also phenobarbital (2, 3), as well as aminoacetonitrile, β-naphthoflavone and pregnenolone-16α-carbonitrile (6) inhibit the critical metabolic route of dimethylnitrosamine leading to its actual carcinogenic metabolite. Kunz et al. (19) reported that administration of phenobarbital inhibits the carcinogenicity of diethylnitrosamine, and Hadjiolov (20) found that administration of aminoacetonitrile inhibits the carcinogenicity of dimethylnitrosamine.

It is well established that β-naphthoflavone inhibits the carcinogenic action of certain hydrocarbons (e.g., 21, 22). It is unknown as yet whether β-naphthoflavone and pregnenolone-16α-carbonitrile inhibit carcinogenesis by nitrosamines, but from the established evidence that they powerfully inhibit the obligatory metabolic route of dimethylnitrosamine, it is expected that they will be found to be potent inhibitors of nitrosamine carcinogenesis. Also, since it has been shown that agents repressing DMN-demethylase synthesis are inducers of other microsomal mixed function oxidases (5, 6), β-naphthoflavone and pregnenolone-16α-carbonitrile can be expected to be inducers of AHH. Moreover neither of these two compounds are carcinogenic, toxic, or appear to have any hormonal or physiological activity. Thus, they show definite promise as effective agents to disrupt the hydrocarbon-nitrosamine pulmonary synergism.

9. Details of experimental design and procedures:

Hydrocarbon-nitrosamine synergism — The syncarcinogenic effects of 3-methylcholanthrene (MC) and dimethylnitrosamine (DMN) will be carried out in male C57BL/6 and DBA/2 mice, 8 weeks old at the beginning of the experiment. Dosage levels and routes of administration will follow those used in mice by Cardesa et al. (9), with the exception that olive oil will be replaced with 1% aqueous methylcellulose as the vehicle for MC (1). Thus mice will receive 6 mg DMN/kg body weight once weekly, injected intraperitoneally as a 0.024% DMN solution in 0.9% saline; MC (as a 0.3% suspension in 1% methylcellulose) will be administered intragastrically at a level of 1.5 mg MC/kg body weight, 3 times weekly; administration of the carcinogens will be for 10 weeks. For each strain (C57BL/6 and DBA/2) of mice the following groups of 60 animals each will be employed: (a) controls receiving the administration vehicles only, (b) mice receiving MC only, (c) mice receiving DMN only, and (d) mice receiving both MC and DMN.

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After the schedules of carcinogen administration will be terminated, the animal groups will be maintained for an additional 10 months (9). Following the routine procedure in our laboratory, during this observational period the animals will be checked twice daily and the state of their health carefully followed. During this observational period any animal showing clear evidence of the presence of a tumor (i.e., large subcutaneous or abdominally localized), or morbidity, will be sacrificed, submitted to complete autopsy, and the tissue samples fixed in neutral formaline for histopathology. All survivors will be sacrificed at the end of the observational period and treated as above. Histopathology will be carried by Dr. Joseph Simon, Professor of Pathology, College of Veterinary Medicine, University of Illinois, Urbana, and by Dr. Cornelia Hoch-Ligeti, Chief, Pathology Service and Research Laboratory, Veterans Administration Center, Martinsburg, W. Va., with whom we have had excellent collaboration for the last 15 years.

Assay of AHH and DMN-demethylase enzyme levels — The level of activity of both enzymes, AHH and DMN-demethylase, will be determined, at 1, 3, 5, 8 and 10 weeks, in liver and lung of mice submitted to the following regimes: (a) C57BL/6 mice receiving MC, (b) C57BL/6 mice receiving DMN, (c) C57BL/6 mice receiving MC + DMN, (d) control C57BL/6 mice receiving the administration vehicles only, (e) DBA/2 mice receiving MC, (f) DBA/2 mice receiving DMN, (g) DBA/2 mice receiving MC + DMN, and (h) control DBA/2 mice receiving the administration vehicles only. Dose levels and routes of administration of the carcinogens will parallel those described for the synergism experiments above.

Appropriate amounts of liver or lung tissue will be pooled for the isolation of microsomes, which will be carried out by the usual method in 0.25 M sucrose (2). In the assay of DMN-demethylase, the demethylation reaction and measurement of HCHO will follow the procedures routinely used in our laboratory (2).

Because in these studies MC will serve a possible dual role, i.e., not only as an "enzyme inducer-repressor" but also as an actual carcinogenic agent, we believe that MC should be employed as substrate in the in vitro determination of AHH activity levels. Thus the total polar MC metabolites formed by isolated microsomal preparations in the standard incubation medium will be measured fluorimetrically by adapting to this hydrocarbon the method of Wattenberg et al. (23) as modified by Conney and Levin (24).

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Anti-carcinogenic effects of β-naphthoflavone and pregnenolone-16α--carbonitrile. — As it has already been described in "Background and brief statement of working hypothesis" these two compounds are powerful inhibitors of the demethylation of DMN, the metabolic route which is the controlling step of tumor induction by this agent. Thus, combined administration of either of these two compounds with DMN and/or MC should decrease their carcinogenicity.

Since both β-naphthoflavone and pregnenolone-16α-carbonitrile are costly compounds, it will be necessary to determine first the lowest dietary levels in the mouse at which saturation of DMN-demethylase inhibition is reached. In a preliminary study in the rat of the dose-dependence of inhibition of DMN-demethylase biosynthesis by in vivo administered pregnenolone-16α-carbonitrile

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we found that 22-25 mg/day/kg body weight is slightly beyond the saturation dose for inhibition. This is also the appropriate dose for β-naphthoflavone to reach saturation level, as indicated by a comparison of our preliminary data with the results of Cantrell and Bresnick (25).

Once the dose level appropriate for the mouse has been confirmed, this level of β-naphthoflavone or pregnenolone-16α-carbonitrile will be incorporated into the diet of mice receiving MC, DMN, or MC + DMN, in the regimes described for the synergism studies. Administration of an inhibitor will start 1 week prior to the beginning of carcinogen administration. As in the synergism experiments, following termination of the administration schedule the animals will be maintained for an additional observational period of 10 months. Maintenance of the animals, autopsies, and histopathology will be as above.

References:

(1) Hoch-Ligeti, C., Argus, M. F., and Arcos, J. C.: "Combined Carcinogenic Effects of Dimethylnitrosamine and 3-Methylcholanthrene in the Rat". J. Natl. Cancer Inst. 40, 535 (1968).

(2) Venkatesan, N., Arcos, J. C., and Argus, M. F.: "Differential Effect of Polycyclic Hydrocarbons on the Demethylation of the Carcinogen Dimethylnitrosamine by Rat Tissues". Life Sci. 7 (Part I), 1111 (1968).

(3) Venkatesan, N., Argus, M. F., and Arcos, J. C.: "Mechanism of 3-Methylcholanthrene-induced Inhibition of Dimethylnitrosamine Demethylase in Rat Liver". Cancer Res. 30, 2556 (1970).

(4) Venkatesan, N., Arcos, J. C., and Argus, M. F.: "Amino Acid Induction and Carbohydrate Repression of Dimethylnitrosamine Demethylase in Rat Liver". Cancer Res. 30, 2563 (1970).

(5) Argus, M. F., Valle, R. T., Venkatesan, N., Buu-Hoi, N. P., and Arcos, J. C.: "Molecular-size-dependent effects of polynuclear hydrocarbons on mixed-function oxidases: possible action on cascade-coupled operons". Proc. 1st Europ. Biophys. Congr. EI/38, 187 (1971).

(6) Arcos, J. C., Argus, M. F., and Buu-Hoi, N. P.: "Repression of dimethylnitrosamine demethylase by polynuclear hydrocarbons and other compounds". Federation Proc. 32, 702 (1973).

(7) Arcos, J. C., Conney, A. H., and Buu-Hoi, N. P.: "Induction of Microsomal Enzyme Synthesis by Polycyclic Aromatic Hydrocarbons of Different Molecular Sizes". J. Biol. Chem. 236, 1291 (1961).

(8) Venkatesan, N., Arcos, J. C., and Argus, M. F.: "Induction and Repression of Microsomal Drug-metabolizing Enzymes by Polycyclic Hydrocarbons and Phenobarbital: Theoretical Models". J. theor. Biol. 33, 517 (1971).

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(11) Wynder, E. L.: "Environmental Carcinogenesis and Cocarcinogenesis". Presented in Pharmacol. Symp. on "Mechanisms of Chemical Oncogenesis". 57th Federation Meet., Atlantic City, 1973, April 17.

(12) Wynder, E. L., and Hoffmann, D.: "Experimental Tobacco Carcinogenesis". Science 162, 862 (1968).

(13) Argus, M. F., and Hoch-Ligeti, C.: "Comparative Study of the Carcinogenic Activity of Nitrosamines". J. Natl. Cancer Inst. 27, 695 (1961).

(14) Smuckler, E. A., Arrhenius, E., and Hultin, T.: "Alterations in Microsomal Electron Transport, Oxidative N-Demethylation and Azo--Dye Cleavage in Carbon Tetrachloride and Dimethylnitrosamine Induced Liver Injury". Biochem. J. 103, 55 (1967).

(15) Thomas, P. E., Kouri, R. E., and Hulton, J. J.: "The Genetics of Aryl Hydrocarbon Hydroxylase Induction in Mice: A Single Gene Difference between C57BL/6 and DBA/2J". Biochem. Genet. 6, 157 (1972).

(16) Nebert, D. W., Goujon, F. M., and Gielen, J. E.: "Aryl Hydrocarbon Hydroxylase Induction by Polycyclic Hydrocarbons: Simple Autosomal Dominant Trait in the Mouse". Nature (New Biol.) 236, 107 (1972).

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(19) Kunz, W., Schaude, G., and Thomas, C.: "Die Beeinflussung der Nitrosamincarcinogenese durch Phenobarbital und Halogenkohlenwasserstoffe". Z. Krebsforsch. 72, 291 (1969).

(20) Hadjiolov, D. : "The Inhibition of Dimethylnitrosamine Carcinogenesis in Rat Liver by Aminoacetonitrile". Z. Krebsforsch. 76, 91 (1971).

(21) Wattenberg, L. W., and Leong, J. L.: "Inhibition of the Carcinogenic Action 7,12-Dimethylbenz[a]anthracene by β-naphthoflavone". Proc. Soc. Exp. Biol. Med. 128, 940 (1968).

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(23) Wattenberg, L. W., Leong, J. L., and Strand, P. J.: "Benzpyrene Hydroxylase Activity in the Gastrointestinal Tract". Cancer Res. 22, 1120 (1962).

(24) Conney, A. H. and Levin, W.: "Induction of Hepatic 7,12-Dimethylbenz(a)anthracene Metabolism by Polycyclic Aromatic Hydrocarbons and Aromatic Azo Derivatives". Life Sciences 5, 465 (1966).

(25) Cantrell, E., and Bresnick, E. : "Evidence for Type II Induction of Microsomal Enzymes by β-Naphthoflavone in Rat Liver Preparations". Life Sci. 10 (Part II) 1195 (1971).

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The Seamen's Memorial Research Laboratory at the U.S. Public Health Service Hospital has a total laboratory area of 4800 square feet, plus air-conditioned animal quarters of a total of 3000 square feet. The applicant has at his disposal approximately 1400 square feet of laboratory space, including a well-equipped cold room, approximately 2400 square feet of animal quarter space, and adequate office space. This is made available to the applicant through the Tulane Department of Medicine.

The major equipment in the applicant's laboratory space consists of the following: a Beckman DB spectrophotometer with recorder, a Shimadzu spectrophotometer with flow dichroism attachment, 4 Coleman spectrocolorimeters, a Photovolt fluorimeter, a Brice-Phoenix light-scattering photometer complete with ratio recorder, an Abbe refractometer, a Rudolph manual spectropolarimeter, 2 fourteen-manometer Warburg apparatuses, a "Yellow Springs" recording oxygen monitor, a high wattage ultrasonic generator, two analytical balances, several pH meters, a semi-automatic tensiometer, a "Spinco L" preparatory ultracentrifuge (including swinging bucket rotor and other accessories for polysome profiling), 3 "Servall" high-speed table centrifuges, 2 heavy-duty International model V centrifuges, a starch-block electrophoresis apparatus with circulating cooling system, a lyophilizing apparatus, 2 heating and cooling precision waterbaths, 2 Dubnoff waterbath incubator shakers, refrigerators, a deep freeze unit, an incubator. The use of a Microtech gas chromatograph and of an osmometer are available in the laboratory.

11. Additional facilities required:

12. Biographical sketches of investigator(s) and other professional personnel (append):

Curricula of three investigators was included with the original application 7/9/73.

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

List of 10 publications see next page.

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13. Publications: (reprints were enclosed with original application 7/9/73).

(1) Argus, M.F., Sohal, R.S., Bryant, G.M., Hoch-Ligeti, C., and Arcos, J.C.: "Dose-Response and Ultrastructural Alterations in Dioxane Carcinogenesis. Influence of Methylcholanthrene on Acute Toxicity". Europ. J. Cancer 9, 237 (1973).

(2) Argus, M.F., Valle, R.T., Venkatesan, N., Buu-Hoi, N.P., and Arcos, J.C.: "Molecular-Size-Dependent Effects of Polynuclear Hydrocarbons on Mixed-Function Oxidases: Possible Action on Cascade--Coupled Operons". Proc. 1st Europ. Biophys. Congr. EI/38, 187 (1971).

(3) Argus, M.F., White, L.E., Bryant, G.M., Arcos, J.C., and Hoch-Ligeti, C.: "Molecular Specificity of the Tumorigenic Action of Ethionine: The Inactivity of S-Ethylcysteine. Action on Respiratory Parameters". Z. Krebsforsch. 75, 201 (1971).

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(6) Hoch-Ligeti, C., Argus, M.F., and Arcos, J.C.: "Induction of Carcinomas in the Nasal Cavity of Rats by Dioxane". Brit. J. Cancer 24, 164 (1970).

(7) Arcos, J.C., Mathison, J.B., Tison, M.J., and Mouledoux, A.M.: "Effect of Feeding Amino Azo Dyes on Mitochondrial Swelling and Contraction. Kinetic Evidence for Deletion of Membrane Regulatory Sites". Cancer Res. 29, 1288 (1969).

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(10) Arcos, J.C., and Simon, J.: "Effect of 4'-Substituents on the Carcinogenic Activity of 4-Aminoazobenzene Derivatives". Arzneim.-Forsch. (Drug Res.) 12, 270 (1962).

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14. First year budget:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Joseph C. Arcos, D. Sc.	20%	
Mary F. Argus, Ph. D.	40%	$ 7,502
Joseph Simon, D. V. M., Ph. D.	10%	
Technical		
Georgia M. Bryant, B. A., Technician V	50%	4,789
Laboratory Technician (to be recruited)	100%	7,367
Laboratory Technician (to be recruited)	100%	7,367
William Hayes, Senior Animal Attendant	33%	2,215
Assistant Animal Attendant (to be recruited)	100%	4,466
	Sub-Total for A	33,706

B. Consumable supplies (by major categories)	
Animals	$ 5,200
Animal feed	2,000
Carcinogens & other Chemical Agents	2,000
Glassware	500
Animal Bedding & cleaning supplies for animal quarters	1,200
Sub-Total for B	10,900

C. Other expenses (itemize)	
Maintenance Contract for Spinco Ultracentrifuge	350
Contribution to maintenance of cold laboratory	400
Contribution to Xerox rental	200
Sub-Total for C	950
Running Total of A + B + C	45,556

D. Permanent equipment (itemize)

Stereoscopic microscope	800
Animal cages*	2,000
Sub-Total for D	2,800
E	6,833
Total request	55,189

E. Indirect costs (15% of A+B+C)

15. Estimated future requirements: (* and † see next page)

	Salaries	Consumable Suppl.	Other Expenses†	Permanent Equip.*	Indirect Costs	Total
Year 2	35,728	9,600	13,350	5,200	8,802	72,680
Year 3	37,872	7,500	13,350		8,808	67,530

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Explanation of specific budgetary items:

* The species in essentially all investigations in this laboratory in the last 12 years was the rat. Guinea pigs have been used to a small extent. Mice, however, have not been used since 1962. For this reason we have very few cages for mice. Consequently, the funds requested in the first year is for the purchase of a sufficient number of mouse cages to begin the experiments. The funds under "Permanent Equipment" in the second year is partly for the purchase of some additional cages, since the second year is the time of maximum occupancy of the facilities for these experiments, when both the enzyme determinations during carcinogen administration as well as the carcinogenesis experiments will be at their peak. The greatest portion of the funds ($4,600) for "Permanent Equipment" in the second year is, however, for the purchase of an inexpensive Beckman DB spectrophotometer. This is an essential expenditure, since the two spectrophotometers we now have (a Beckman DB double-beam and a Shimadzu QV-50 single beam) are extensively tied up with ongoing experiments. Moreover, our Beckman DB is a 13 year-old instrument requiring servicing more and more often, which means substantial interruptions in the work. — We computed that the serial enzyme determinations will require the use of a spectrophotometer 60-80% of the time for at least $1\frac{1}{2}$ years, considering the number of groups and the experiments to be carried out in statistically analyzable numbers.

† The category "Other Expenses" in the second and third year represents mainly the expenditures for the histopathological examination of the slides resulting from the carcinogenesis studies. The sums in this category also include the standard yearly expenditures for the items listed in "Other expenses" in the first year.

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Faculty Research Award (exclusively salary grant for Joseph C. Arcos)	American Cancer Society	127,887	1/1/72 - 12/31/76
Pharmacology of Repressible Mixed--Function Oxidases	NCI CA-13206	111,626	1/1/72 - 12/31/74

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Pharmacology & Biochemistry of the Oncogen, Dioxane	NCI CA-15111 (NIEHS is also considering funding this application)	384,390	9/1/73 - 8/31/78 (not yet activated as of 1/1/74)
Molecular Bases of the Oncogenic Action of Dioxane	American Cancer Society	139,426	7/1/74 - 6/30/77

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal Investigators: Date of Signatures: 1/17/74

Typed Name Joseph C. Arcos and Mary F. Argus

Signature Joseph C. Arcos Mary F. Argus

Telephone (504) 899-3441 331
Area Code Number Extension

Checks payable to

Tulane University

Mailing address for checks

Jesse B. Morgan, Comptroller

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